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Critical agitation for microcarrier suspension in orbital shaken bioreactors: Experimental study and dimensional analysis

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HIGHLIGHTS

- Experimental characterization of particle suspension in orbital shakers.
- New correlation predicting critical agitation conditions for complete particle suspension.
- Larger orbital diameters promote smaller power dissipations at complete particle suspension.

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ABSTRACT

Orbital shaken bioreactors are widely used at the laboratory scale for the culture of animal cells in suspension mode. In the case of adherent-dependent cell lines, the choice of agitation conditions at which all particles are just-suspended (or attain complete suspension) has often to be determined. Indeed, with orbital shaken bioreactors, this choice results from the combination of two parameters: the orbital diameter and the agitation rate. That is why, a new experimental protocol for the determination of critical agitation conditions for microcarrier complete suspension has been developed in this paper. It consisted in a side-view visualization of blue-stained particles in shaken Erlenmeyer flasks and cylindrical flasks. 220 experiments representative of animal cell culture conditions have been carried out to study the effect of various operating conditions (bioreactor size and geometry, particle type, density and diameter, liquid viscosity, shaking diameter, filling ratio). Furthermore, a dimensional analysis has been performed, leading to a correlation relating a Froude number (in which the critical agitation N_c for complete particle suspension is embedded) to four other dimensionless numbers. Then, the critical agitation conditions determined in this paper were analyzed and discussed with respect to the data available in the literature on the flow structure occurring inside the flask. Our findings revealed that high orbital shaking diameters and large cylindrical vessels should be promoted to get microcarriers into suspension at a minimized power dissipation per unit of volume.

1. Introduction

Adherent-dependent cell culture on microcarriers in mixed bioreactors finds many applications in the pharmaceutical industry for the production of vaccines. Other applications are now found in tissue engineering and in cell therapy, particularly for the expansion of mesenchymal stem cells (Ferrari et al., 2012). In adherent-dependent cell cultures, a particular attention has to be paid to bioreactor agitation. A too low agitation condition may lead to microcarrier settling and to the occurrence of some gradients of pH, O_2 or nutrient concentrations whereas a too vigorous mixing would lead to cell or microcarrier damage. In microcarrier cultures, hydromechanical damage arises from particle–particle collisions, impeller–microcarrier interactions and/or liquid turbulence and, especially, from turbulent eddies whose characteristic dimension is similar to microcarrier size (Cherry and Papoutsakis, 1988). Microcarrier suspension in bioreactors is generally looked for in order to avoid particle–particle contact points, and thus to increase the available adherence area for the cells and to enhance mass transfer between cells and their environment.

Hence, to define a suitable mixing strategy as well as for scale-up purposes, the critical agitation rate for particle complete

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suspension N_c (also called the just-suspended agitation rate) has to be considered and quantified: N_c refers to the minimum agitation conditions at which all particles attain complete suspension (Zwietering, 1958). The definition of the critical agitation rate for particle complete suspension relies on usual concepts in solid–liquid mixing tanks. While at low agitation rates, all particles remain settled at the bottom of the vessel, an increase of N induces the motion of a fraction of particles (partial suspension). A further increase of N reduces the number of particles settled till N_c for which no particles remain deposited more than one or two seconds approximately (complete suspension). For higher agitation rates, gradients of particle concentration are reduced towards full homogeneity (homogeneous suspension). Particle suspension is the result of complex phenomena involving turbulent eddies, forces acting on particles (drag, buoyancy, gravity), wall–particle and particle–particle interactions.

In the case of mechanically stirred bioreactors with a central impeller rotating around a vertical axis, the critical agitation rate N_c is generally predicted using the well-known Zwietering equation (Zwietering, 1958) as a function of impeller diameter D, liquid physico-chemical properties (kinematic viscosity ν_L and density ρ_L), particle diameter d_p, density ρ_p and mass concentration of particles X:

$$N_c = S \cdot \nu_L^{0.1} \cdot \left[\frac{g(\rho_p - \rho_L)}{\rho_L}\right]^{0.45} \cdot X^{0.13} \cdot d_p^{0.2} \cdot D^{-0.85} \quad (1)$$

The value of the constant S is related to the geometry of the vessel and impeller. However, Ibrahim and Nienow (2004) have shown that, in the case of microcarrier suspension, the relation (1) may provide a 50% overestimation of N_c, probably due to the low density difference between liquid and microcarriers. Collignon et al. (2010) got further insight into microcarrier suspension by analyzing the liquid flow structure using Particle Image Velocimetry (PIV) measurements in a mechanically stirred vessel. Indeed, they compared the flow induced by seven designs of impellers, chosen among usual designs in animal cell culture processes and especially measured the power dissipation at the critical impeller agitation rate for microcarrier complete suspension. With this approach, the authors identified the TTP Mixel® and the Ear Elephant as two impeller designs minimizing the hydromechanical stresses encountered when complete suspension was reached. Furthermore, these authors showed that, for all impellers, the liquid velocity fields were globally similar at the critical agitation rate for particle complete suspension. Bioreactors agitated by an axial rotation of the impeller remain the most used systems for adherent-dependent cell cultures, especially because detailed knowledge can be found on their global characteristics (mixing time, hydromechanical stress, oxygen-liquid mass transfer), thus allowing a good scalability. Nevertheless, a major issue may arise from the use of air or oxygen sparging to ensure a sufficient amount of dissolved oxygen for cell respiration as it may lead to undesired foam or cell damage due to bubble burst at the gas-liquid free surface (Nienow, 2006).

Besides these usual culture systems, flasks shaken by an orbital motion have been revisited these last years for microbial and cell culture. Indeed, Büchs (2001) has estimated that more than 90% of all culture experiments in biotechnology are performed in shaken bioreactors. More recently, disposable bioreactors for animal cell culture in suspension mode, consisting in a cylindrical vessel shaken by an orbital motion, were shown as valuable systems in terms of mixing efficiency and oxygen transfer up to a scale of 1000 L (Stettler et al., 2007; Tissot et al., 2010; Zhang et al., 2009). An additional advantage of this mode of culture is the lack of damaging bubble bursts. Despite these new developments, surprisingly, concerning animal cell cultures on microcarriers, no data or correlation providing the critical agitation rate for complete suspension of microcarriers or, more generally of any kind of particles, are reported in the literature for shaken bioreactors.

In mechanically stirred vessels, the classic procedure to investigate liquid–solid suspensions consists in observing the vessel bottom. Although it has been intensively studied in the literature, the definition of particle suspension in these systems is still discussed (Zlokarnik, 2001). In shaken flasks, no standard protocol exists until now. As the shaking table is placed under the flask, the visualization of flask bottom would have required a modified shaking table, which would have been more complicated to handle. Therefore, the present study was devoted to investigate the microcarrier suspension in shaken flasks and, especially, to determine the critical agitation conditions (resulting from combined effects between agitation rate and orbital diameter) for microcarrier complete suspension as a function of the operating parameters (density of fluids and particles, viscosity, particle diameter and type). For that, a new protocol was first proposed to experimentally determine N_c for a given orbital shaking diameter. It was based on visual observations of blue-stained particle suspension obtained for 220 mixing conditions, changing flask design (Erlenmeyer flask or cylindrical flask) and size, liquid volume V_L, orbital shaking diameter d_0, liquid viscosity μ_L and density ρ_L, particle type, density ρ_P and diameter d_P. Most of the operating conditions were characteristic of animal cell culture at the laboratory scale. Some additional conditions were also considered to test the robustness of the model, for example by considering other solid particles than the microcarriers used for cell culture. Secondly, a dimensional analysis based on the knowledge of the physical mechanisms involved was performed, leading to a correlation relating a Froude number (in which the critical agitation rate N_c for particle complete suspension was embedded) to four other dimensionless numbers (including the ratio between the orbital shaken diameter and the flask diameter). Then, the critical agitation rates reported in this paper were analyzed and discussed with respect to the data available in the literature on the flow structure occurring inside the flask. At last, our results were used to identify the agitation conditions that promoted microcarriers suspension at a minimized power dissipation per unit of volume.

2. Material and methods

2.1. Shaken flasks and shaker

A bench top shaker Kühner LT-X placed in a temperature and CO_2 partial pressure controlled incubator was used (Kühner, Basel, Switzerland). The orbital shaking diameter d_0 was chosen equal to 1.25, 2.5 and 5 cm. Precisions of agitation rate and temperature measurements were ± 0.1 rpm and ± 0.3 °C respectively. Two geometries and various scales of shaken flasks were used. The first shape was widespread Erlenmeyer flask with a maximum liquid working volume V_T of 125, 250, 500, 1000 mL (Corning, USA) and 5000 mL (Duran, Germany). These systems have convex bottoms (Fig. 1). The second one consisted of glass straight cylindrical vessel with inner diameters of 2.4, 3.8, 4.5, 5.5, 8.7 and 11.5 cm. A schematic representation and detailed dimensions of Erlenmeyer flasks are given in Fig. 1 and in Table 1.

From Table 1, it can be observed that a degree of homothety existed between the Erlenmeyer flasks of different sizes. In particular, the following ratio was conserved whatever the flasks:

$$\frac{h_1}{d} = 1.17 \pm 0.07 \quad (2)$$